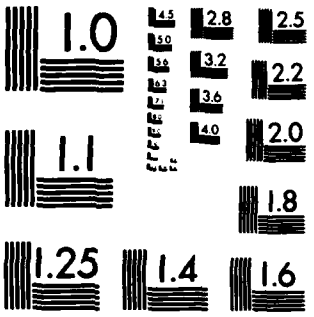
AD-A127 068 DEVELOPMENT OF MONOCLONAL ANTIBODIES TO TRYPANOSOMA BRUCEI RHODESIENSE ANTIGENS(U) NEW MEXICO UNIV ALBUQUERQUE DIV OF TROPICAL AND GEOGRAPHIC ME.. 1/1

UNCLASSIFIED G H CAMPBELL 28 FEB 82 DAMD17-79-C-9127 F/G 6/5 NL

END
DATE FILMED
5 83
DTIC




MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

Report Number 2

# DEVELOPMENT OF MONOCLONAL ANTIBODIES TO T. B. RHODESIENSE ANTIGENS

Annual Report

Gary H. Campbell, Ph.D.

28 February 1982

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD 17-79-C-9127

University of New Mexico Medical Center
Albuquerque, New Mexico 87131

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

# REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

| | |
|---|---|
| 1. REPORT NUMBER: 2 | 2. GOVT ACCESSION NO.: AD-A127068 |
| 3. RECIPIENT'S CATALOG NUMBER: | |
| 4. TITLE (and Subtitle): Development of Monoclonal Antibodies to Trypanosoma brucei rhodesiense Antigens | 5. TYPE OF REPORT & PERIOD COVERED: Final Report |
| | 6. PERFORMING ORG. REPORT NUMBER: |
| 7. AUTHOR(s): Gary H. Campbell, PhD | 8. CONTRACT OR GRANT NUMBER(s): DAMD 17-79-C-9127 |
| 9. PERFORMING ORGANIZATION NAME AND ADDRESS: Division of Tropical and Geographic Medicine, UNM Med. Center/ 943 Stanford Drive, N.E., Albuquerque, New Mexico 87131 | 10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: |
| 11. CONTROLLING OFFICE NAME AND ADDRESS: | 12. REPORT DATE: 28 February 1982 |
| | 13. NUMBER OF PAGES: |
| 14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office): | 15. SECURITY CLASS. (of this report): |
| | 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE: Unclassified |

16. DISTRIBUTION STATEMENT (of this Report)

Unlimited

DISTRIBUTION STATEMENT A
Approved for public release;
Distribution Unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

same

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Trypanosoma b. rhodesiense, African trypanosomiasis, Monoclonal Antibodies, Hybridoma.

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

This report, covering work performed from 1 September 1979-28 February 1982, describes methodology of tissue culture, hybridization procedures, immunization schedules and antibody assays developed to produce monoclonal antibodies to variant specific surface antigens of Trypanosoma b. rhodesiense organisms of the Walter Reed Army Trypanozoon antigenic type (WRATat) serodeme. Monoclonal antibodies have been produced to WRATats 2-19. These reagents will be critical in assessment of antigenic variation and in potential vaccine development programs.

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT

This contract was porposed to produce monoclonal antibody reagents that could be used to identify individual antigenic variants of Trypanosoma b. rhodesiense organisms. Variant specific monoclonal antibodies can be used in a variety of studies concerning antigenic variation.

The first efforts on the contract were to establish a monoclonal antibody production laboratory and to establish assay techniques to be used as screening procedures for the antibodies. This was accomplished with the production of antibodies to WRATat 3 and 5 and reported in the attached annual report.

The established techniques were then applied in a routine manner to produce antibodies to other antigenic types. A listing of the reactivities of the various monoclonal antibodies and the variant types to which they react is shown in the attached copy of a publication. In addition to these, antibodies were produced to WRATats 9, 10, 11, 15, 17, and 18.

The cell lines producing these antibodies have been sent to Klaus Esser, Department of Immunology, WRAIR. Duplicate specimens will be maintained by Dr. Campbell at the Centers for Disease Control in Atlanta, GA. Antibodies from selected lines have been distributed for collaborative experiments to Dr. Terry Pearson, University of Victoria, and Dr. George Cain, University of Iowa.

The production of some of the monoclonal antibodies in this contract has been described in the following publication (attached):

Gary H. Campbell, Susan Griswold, George Cain, Janis Giorgi, and Noel L. Warner. Monoclonal Antibodies to Antigens of T. Rhodesiense. In Monoclonal Antibodies and T Cell Hybridomas, P. 323. , Eds. G. J. Hammerling, U. Hammerling, and J. F. Kearney. Elsevier/ North Holland, 1981

# 6.3.4. Monoclonal antibodies to antigens of *Trypanosoma rhodesiense*

GARY H. CAMPBELL, SUSAN GRISWOLD, GEORGE CAIN, JANIS V. GIORGI and NOEL L. WARNER

*Division of Tropical and Geographic Medicine, and Immunobiology Laboratories, University of New Mexico School of Medicine, Albuquerque (New Mexico, U.S.A.)*

## Introduction

Infection of a host with African trypanosomes can result in a variety of chronic, immunologically mediated consequences, including polyclonal stimulation, immunosuppression, and immunopathological lesions[1]. A major factor in the failure of the humoral antibody response to control the infection is the ability of the organisms to undergo extensive changes in their glycoprotein surface antigens resulting in a new wave of parasites which are not susceptible to the previously induced antibody responses[2]. It is therefore of considerable practical and basic research interest to determine the mechanisms of such antigenic variation. One aspect of this is the need to clearly define the various internal and external trypanosome antigens of a range of variant types, and this can be best approached by the use of hybridoma technology.

Monoclonal antibodies have now been produced which react with surface components of organisms of different antigenic types, as well as to various internal structures of the organisms. The eventual goal of this work is to use such monoclonal antibodies to characterize trypanosomal antigens which may play a role in the immunopathology of disease and to provide monospecific reagents to identify organisms of specific variable antigen types (VATs).

## Methods

The organisms used for these studies were derived from clones of different antigenic types of *Trypanosoma rhodesiense* of the Walter Reed Army Trypanozoon antigenic type (WRATat) serodeme. WRATat 1–13 are clones produced as previously

described[4]. WRATats 14–19 are clones of trypanosomes derived by Klaus M. Esser, and are serologically distinct from the original 13 WRATats. All clones of specific VATs were stored as stabilates in 10% glycerol at –70°C. Organisms were passed for 4 days in irradiated (750 R) mice before isolation from blood on DE52 columns or before use as an inoculum for non-irradiated mice.

Immunization of C57BL/6J mice for the fusion experiments was performed using a protocol obtained from Klaus Esser by infecting mice intraperitoneally with $10^6$ organisms of a specific VAT, and giving 0.5 mg of the drug Berenil on day 10 to cure the infection. From 2 weeks to 4 months later, the mice were injected intravenously with 0.1–0.3 mg of an antigen preparation enriched for the variant specific antigen[4].

P3/X63-Ag8 BALB/c myeloma cells were maintained in log phase growth in Dulbecco's minimal essential medium (DMEM) with 10% fetal calf serum (FCS), and were fused with immune spleen cells taken 3 days after the second antigen injection, using the hybridoma fusion protocol of Kennett et al.[4]. Briefly, $10^7$ X63 cells were fused with $10^8$ immune splenic lymphoid cells in the presence of polyethylene glycol ($M_r = 1000$). Cells were pelleted by centrifugation and resuspended in 30 ml DMEM containing hypoxanthine and thymidine; aminopterin was added 24 h later. Cells were plated into approximately 500 microtiter wells (96 wells/plate). Hypoxanthine–aminopterin–thymidine (HAT) medium was changed in the wells twice a week. When cell growth was visible at about 10 days, the medium was changed twice in one day. Supernatants were tested for antibody 3 days later. Cells in antibody-positive wells were cloned by limiting dilution using thymocytes from BALB/c mice as feeder cells. Supernatant from wells containing growing clones was again tested for antibody activity. Antibody-producing cells were then frozen in 10% DMSO and stored in liquid nitrogen. Cell lines were numbered according to fusion number (WRATat used for immunization), well number and letter of the clone well.

Antibody assay of the supernatants was performed by an indirect fluorescent antibody assay using a mixture of FITC rabbit anti-mouse 7 S globulins and anti-mouse $\mu$-chain (Meloy Laboratories) as the second step reagent. Antigen slides were made as a thin film preparation of trypanosomes isolated by DE-52 columns from the blood of infected mice[4]. Organisms were suspended in 50% FCS in phosphate-buffered saline. Slides were made from suspensions of organisms containing a mixture (75%/25%) of two antigenic types. Supernatants from a fusion experiment using (for example) WRATat 3 immune cells, were tested against slides containing 75% WRATat 3 and 25% of another WRATat type. Thus, when some organisms were positive and some were negative, variant antigenic specificity could be inferred. When all organisms were labeled, a non-variant specific type antigen was presumably involved – usually an internal antigen.

## Results

The primary objective of this work was to produce monoclonal antibody reagents to a variety of the available antigenic types of *T. rhodesiense* organisms of the WRATat serodeme. To this end, 50 fusion experiments have been performed. In some cases, cell lines producing antibody to the desired variable antigen type specificity were derived from one experiment. In other cases, repeated fusions were required. In many cases, several VAT-specific antibody-producing cell lines were derived. The redundant cell lines were frozen for later use. In most experiments, antibody reacting against non-variant specific antigens was detected in addition to the variant specific reactions.

The distribution of antibody against variant and non-variant specific antigens in three representative fusion experiments is shown in Table 1. The proportion of trypanosome-reactive cell lines was extremely variable from experiment to experiment.

The appearance of the variant specific reaction is shown in Fig. 1. The slide used in this assay contained 75% WRATat 14 and 25% WRATat 11 organisms. Ap-

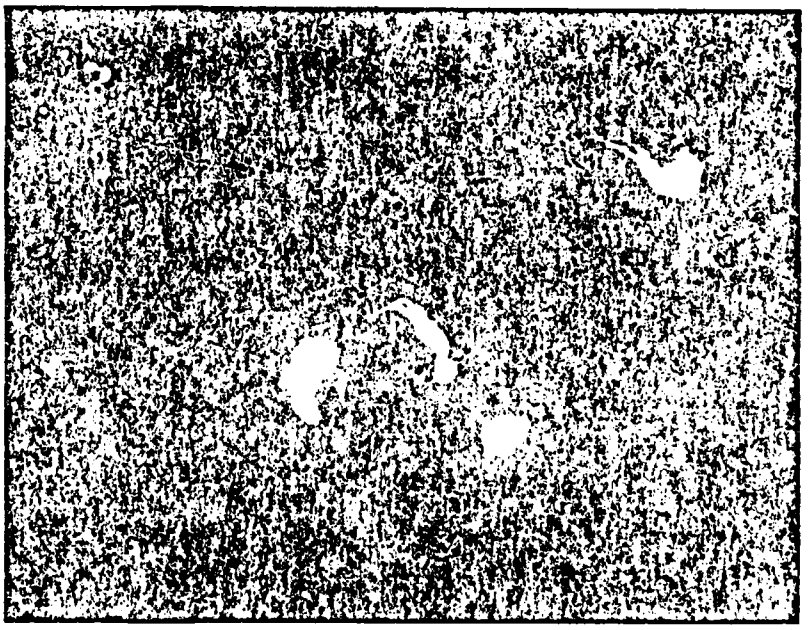


Fig. 1. Variant-specific fluorescence reactivity of monoclonal antibody 21(14)146D. The slide contained a mixture of 75% WRATat 14 and 25% WRATat 11 *T. rhodesiense* organisms. Note several unlabeled organisms (presumed to be WRATat 11 type).

TABLE 1

Trypanosome-specific reactivity in individual fusion experiments

| Fusion No. (WRATat type) | No. wells growing/ No. wells plated | No. wells with anti-trypanosome antibody | |
|---|---|---|---|
| | | Variant-specific | Non-variant-specific |
| 9 (5) | 333/912 | 16 | 63 |
| 12 (12) | 83/960 | 3 | 4 |
| 21 (14) | 349/828 | 38 | 22 |
| 50 (2) | 159/384 | 3 | 2 |

TABLE 2

Specificity of prototype anti-*T. rhodesiense* hybridoma cell lines

| Clone No. | Specificity |
|---|---|
| 50(2)103B[a] | WRATat 2 |
| 7(3)27D | WRATat 3 |
| 9(5)137Q | WRATat 5 |
| 25(6)17C | WRATat 6 |
| 43(7)57G | WRATat 7 |
| 19(8)94E | WRATat 8 |
| 12(12)8D | WRATat 12 |
| 40(13)27I | WRATat 13 |
| 21(14)146D | WRATat 14 |
| 36(16)57F | WRATat 16 |
| 31(19)20B | WRATat 19 |
| 21(14N)109A[b] | NVS-even fluorescence pattern (panel 2B) |
| 23(2N)67F | NVS-central fluorescence (2nuclear) (panel 2F) |
| 21(14N)59L | NVS-posterior fluorescence (panel 2D) |
| 32(19N)36D | NVS-granular line (panel 2E) |
| 23(2N)140D | NVS-flagellar (panel 2C) |

[a] For example, 50(2)103B represents experiment No. 50 prepared against WRATat 2, with prototype clone being well 103 clone B.

[b] N refers to non-variant-specific reactivity.

proximately 75% of the organisms fluoresced when tested against culture supernatant 21(14)146D which was derived from a fusion using WRATat 14 organisms as immunogen. This supernatant was not reactive when tested against any of the other WRATats, and this variant-specific type behavior was used to characterize all the other VAT monoclonal reagents obtained (Table 2).

The monoclonal antibodies produced against non-variant-specific antigen showed

several distinct patterns of immunofluorescence as shown in Fig. 2. Panel A shows a variant-specific reaction as distinguished by the non-fluorescing heterologous antigenic type. The same even fluorescence is seen in panel B, however *all* the organisms fluoresce, indicating non-variant-specific reactivity. Fluorescence of the flagellum is shown in panel C. Panel D shows non-variant specific fluorescence in an elongated pattern in the posterior portion of the trypanosomes. This pattern varied in the size of the elongated area of fluorescence from organism to organism and from one monoclonal antibody to another. It was the most abundant of the non-variant-specific patterns observed, being usually detected at least once in most fusion experiments. Panel E shows a slightly granular pattern of fluorescence, occurring as a line opposite the flagellum. Reactivity as shown in panel F may be associated with the nucleus.

Reagents have now been produced which allow detection of specific trypanosome antigenic types in dried blood smears containing unknown antigenic types of organisms. Table 2 lists the prototype monoclonal reagents and their specificity that have been produced. To date, variant-specific monoclonal antibody has been produced to 11 antigenic types of the WRATat serodeme of *T. rhodesiense*.

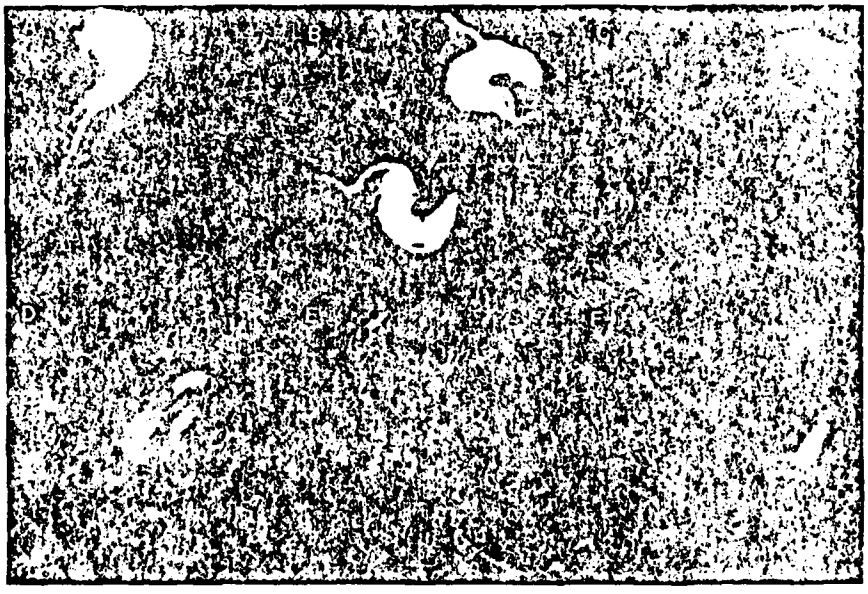



Fig. 2. Patterns of reactivity of monoclonal antibodies against *T. rhodesiense*. The slide contained a mixture of 75% WRATat 14 and 25% WRATat 11 trypanosomes. A shows variant-specific reactivity of 21(14)146D. B–F show different patterns of non-variant-specific reactivity as described in the text using prototype reagents listed in Table 2

## Discussion

This work has demonstrated the ability to produce monoclonal antibodies which identify variant-specific antigens of several antigenic types of *T. rhodesiense* organisms. In addition, the use of organisms prepared as a dried smear in an indirect fluorescence assay has allowed the detection of monoclonal antibodies directed against various morphological structures of the parasite.

The production of this range of hybridomas has been accomplished using mice that were infected, cured, and boosted with trypanosomal antigens eluted from the trypanosomes. This circumvented the necessity for purified variant surface antigens as used by Pearson et al. [6,7]. The production of monoclonals having several different reactivity patterns presumably relates to the use of heterogeneous antigen preparation.

These reagents will be useful in a variety of studies involving the WRATat organisms. A prime usage will be to identify the VATs present in a smear of infected blood. In this way the process of antigenic variation may be more definitively monitored during chronic infection and thus help to uncover the complexities of the host–parasite relationship.

## Acknowledgements

This work was supported by contract No. DAMD-17-79-C-9127 from the U.S. Army Medical Research and Development Command.

## References

1 Mansfield, J.M., 1978, Cell. Immunol. 39, 204.
2 Cross, G.A.M., 1978, Proc. R. Soc. London B 202, 55.
3 Campbell, G.H., K.M. Esser, B.T. Wellde and C.L. Diggs, 1979, Am. J. Trop. Med. Hyg. 28, 974.
4 Kennett, R.H., K.A. Denis, A.S. Tang and N.R. Klinman, 1978, in: Current Topics in Microbiology and Immunology, eds. F. Melchers, M. Potter and N.L. Warner (Springer-Verlag, New York) Vol. 81, p. 77.
5 Lanham, S.M. and D.G. Godfrey, 1980, Exp. Parasitol. 28, 521.
6 Pearson, T.W., M. Pinder, G.E. Roelants, S.K. Kar, L.B. Lundin, K.S. Mayor-Withey and R.S. Hewett, 1980, J. Immunol. Methods 34, 141.
7 Pearson, T. and L.A. Anderson, 1980, Anal. Biochem. 101, 377.